Abstract No. Laws417

Crystal Structure of trp Aporepressor Point Mutant Leu-->Phe 75

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Beamline: X12C

Introduction:The L75F trp repressor (TrpR) point mutation confers a temperature-sensitive repression phenotype, and displays global biophysical changes not expected for a simple hydrophobic residue substitution (1). The crystal structure of the mutant was determined in order to investigate the structural basis for these changes.

Methods and Materials: Thin plate like crystals of L75F Trp-apo repressor were grown by vapor diffusion over a reservoir of 30% PEG4000, 100mM Tris pH 8.5, and 200mM MgCl2. Crystals trays were transported to the NSLS and transferred to a single solution of cryoprotectant containing the above components plus 20% glycerol for about 30 seconds, then flash cooled to 100K on a nitrogen cryostream. The wavelength of incident radiation was set to 1.0 Angstrom.

Results: The crystals were determined to have space group symmetry P2(1) and unit cell dimensions of a=36, b=54 c=56 Angstrom, beta=100°, with one biological dimer per asymmetric unit. Native diffraction data were collected from a single crystal to 2.4 Angstrom at X12C. The structure was solved by molecular replacement and has been refined to R-free = 27.9% and R = 22.3%.

Conclusions: One subunit of the two fold-symmetric structure strongly resembles wild type *trp* aporepressor. The other subunit has a large deformation of the E-helix, located about ten residues away from the L75F mutation. The N-terminal half of the E-helix is displaced by a registration shift of three residues. This structure demonstrates that point mutations can have significant, global effects on protein conformation.

Acknowledgments: This work has been funded by NIH R01-GM021589 to H.M.B. We thank Michael Becker, Anand Saxena, Dieter Schneider, Bob Sweet, and their colleagues for assistance at the NSLS protein crystallography beamlines.

References: 1. Jin, L., Fukayama, J.W., Pelczer, I. & Carey, J. Long-range effects on dynamics in a temperature-sensitive mutant of *trp* repressor. *J Mol Biol*., **285**, 361-78. (1999).